# RECONCILING PILLARS OF TRANSIENT GENE EXPRESSION:

## FROM DNA PREP VIA MEDIA, REAGENT AND CELL LINE DEVELOPMENT TO HOLISTIC PROCESS OPTIMIZATION

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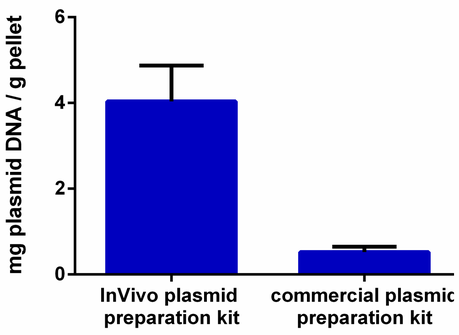
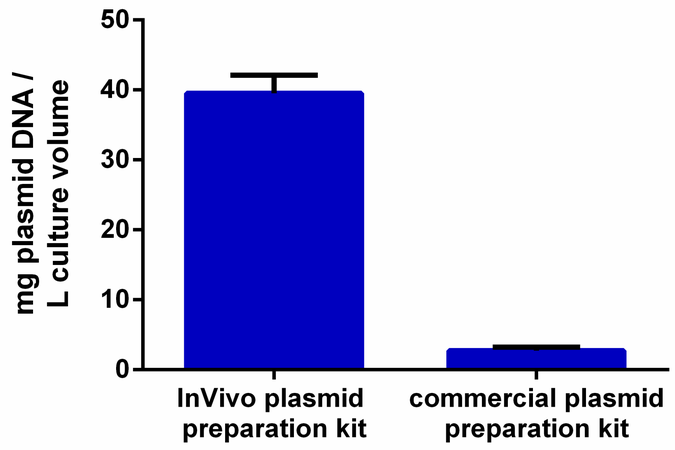
During the last years, InVivo BioTech Services has realized a novel technology for efficient transient transfection and expression in HEK and CHO cells. In the process of transient gene expression, introduction of the gene of interest into the host cell can be performed by various physical, chemical or biological methods. Because of the greater scalability compared to physical methods and no safety concerns or restrictions that are associated with the use of viral systems, a transfection using chemical methods is the method of choice. However, up to now up-scaling is limited by various scientific and economic bottlenecks regarding plasmid preparation and vector design, transfection reagents, host cell lines and cultivation media. To overcome these bottlenecks, InVivo BioTech Services developed in cooperation with emp Biotech, Berlin, and Xell AG, Bielefeld, a transfection reagent and a new culture medium that can be used for transfection and production. The in-house establishment of a TGE optimized HEK cell line and a method for large-scale plasmid preparation completed the production platform for HTS approaches and large-scale transfection for the production of gram quantities IgG within days.

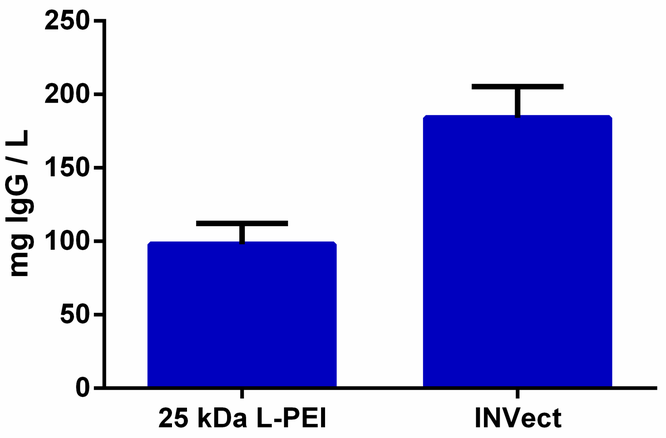
## DNA Preparation

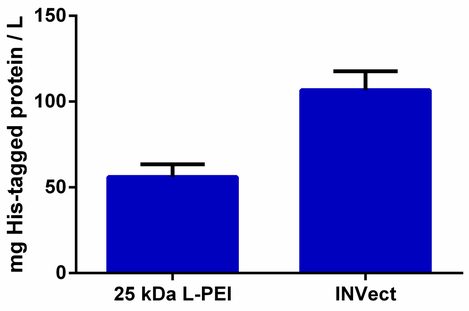



Several *E.coli* strains and media were screened for high productivity, high quality and flexibility for DNA preparation in comparison to commercial kits. Additionally a purification process was implemented using a anion exchanger. Up-scaling this process results in approx. 250 mg purified plasmid DNA. Additionally, vector design indicates that a high yielding TGE process can be achieved by “minimizing” the vector backbone.

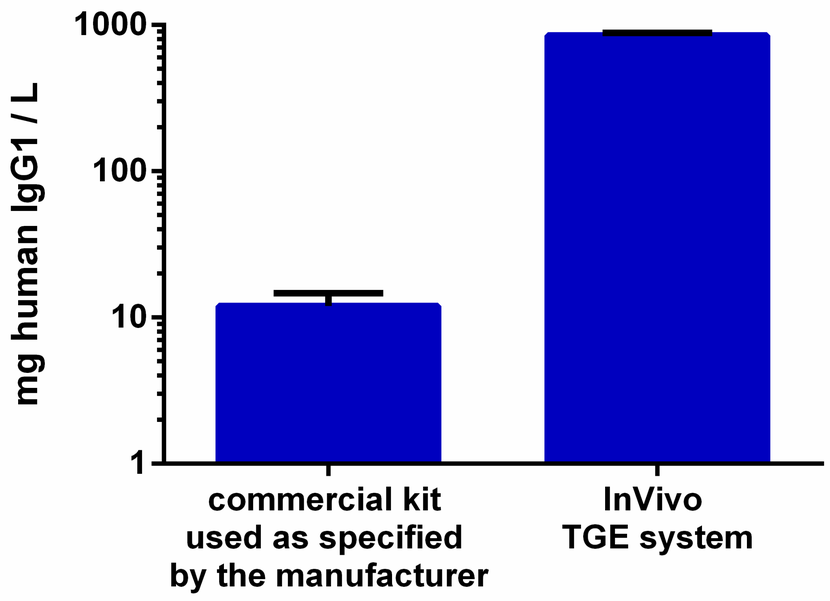
## Transfection Reagent




INVect is a transfection reagent which demonstrates low cell toxicity for transient transfection of mammalian cells and delivers extremely high transfection efficiencies up to 90%, 24h post transfection. The use of INVect for transfection under TGE conditions leads to exceptionally high levels of protein expression and outperforms 25kDa linear PEI by 2-fold.

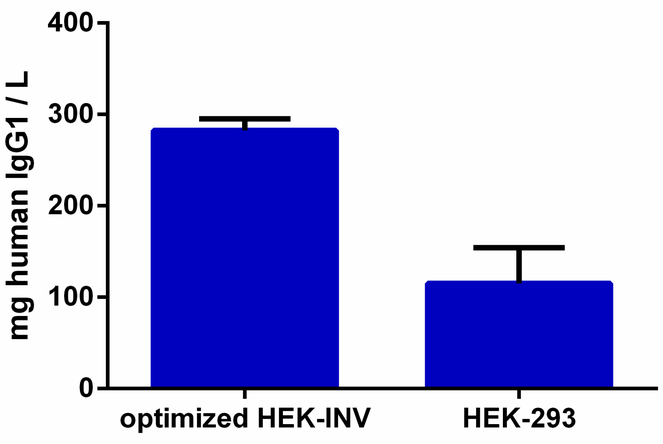
By combining these set screws, it was possible to generate a high yielding TGE production process. A holistic DoE-based optimization of all relevant parameters resulted in about 80-fold increase in human IgG1 production in comparison to a commercial available TGE system.

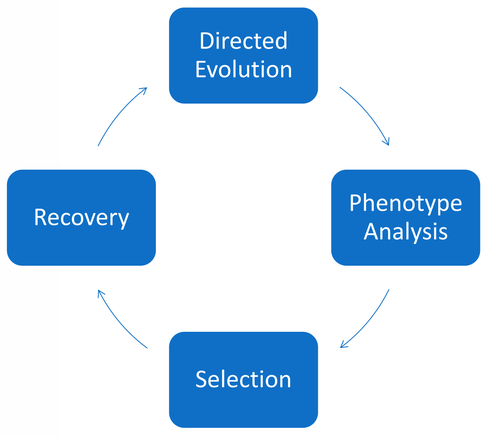



In conclusion, InVivo’s TGE system includes an optimized and advanced cell line as well as vector system, a novel transfection reagent and a specially designed media. This allows high throughput production of recombinant proteins for early development and lead identification as well as gram-scale production for pre-clinical trials.

## Cell Line

To generate an optimized host cell line for TGE processes we utilized a directed evolution approach, which results in a threefold increase in IgG productivity. For this purpose, an iterative process of evolution rounds followed by metabolomic phenotype analysis and selection of cells was performed.




## Transfection Medium

Starting from a basal medium we were able to generate a novel medium, which supports high titer transient gene expression. Improvements were achieved by stepwise screening and optimization of media ingredients with regards to higher cell growth transfection efficiency and productivity, resulting in a 4-fold increase in comparison to a reference medium. Furthermore, the new medium supports cell growth and easy adaption to suspension of various parental and prducer HEK cells.